June 20, 2016

Notes on NEGEM Presentation

- Next slide should explain what rice crispr is
- Background slides before defending
- In text citations

sgRNA is produced by RNA Pol III
mCherry on separate promotor
Transient transfection in HEK 293T
rtTa tetracyclin repressor

Outreach activities
- ~3 slides
- Say that we have notes to share with W&M and will put on wiki
- Add in touch tomorrow slides, pictures, graphs

Add acknowledgements

Wednesday 3pm run through slides

Draft e-mail to UMASS Zhang, CRISPR Technologies, Team at Harvard/MIT
Start stable integration
Transform and make mini prep
Transfect + add antibiotic
What We Did Today
- Split Cells
- TouchTomorrow Data
- NEGEM Slides
- Transformation for interlab study

Transformation Protocol for interlab study
- Used Plate 2- cell 6F- Bba-R0040 (Cam^r)
  - 10 microliter water and pipetted up and down, put in microcentrifuge tube
  - 1 microliter pRetro on (amp^r)
- Used 50 microliters of dh5a cells and added all of the above to 200 microliters of LB
- Capped tubes and let shake for an hour and a half
- Plated cells and left overnight

GM, JW
Worked on NEGEM PowerPoint

June 21, 2016

What we did today:

- NEGEM Slides
- Interlab study:
  - Checked transformation
  - Made liquid cultures

Liquid Cultures:

- Chose 6 cultures from the pRetro Plates, avoided major satellite cells
- 5mL LB
- 2.5 microliters Ampicillin
- Added cultures to 15 mL conical tube
- Put them in the shaking incubator

GM, JW
Split cell culture (large flask), 1:5

June 22, 2016

What We Did Today:

- Finished NEGEM presentation
- Looked up people to contact for Integrated Practices
- Practiced presentation for NEGEM

Mini Prepped P-retro

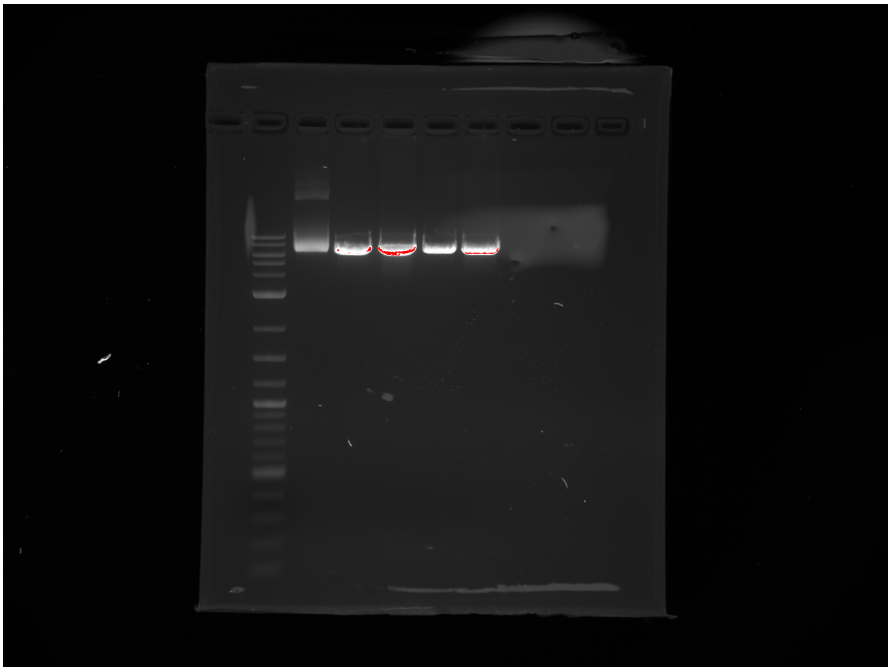
June 23, 2016

What We Did Today:

Ran Gel with Restriction Enzymes

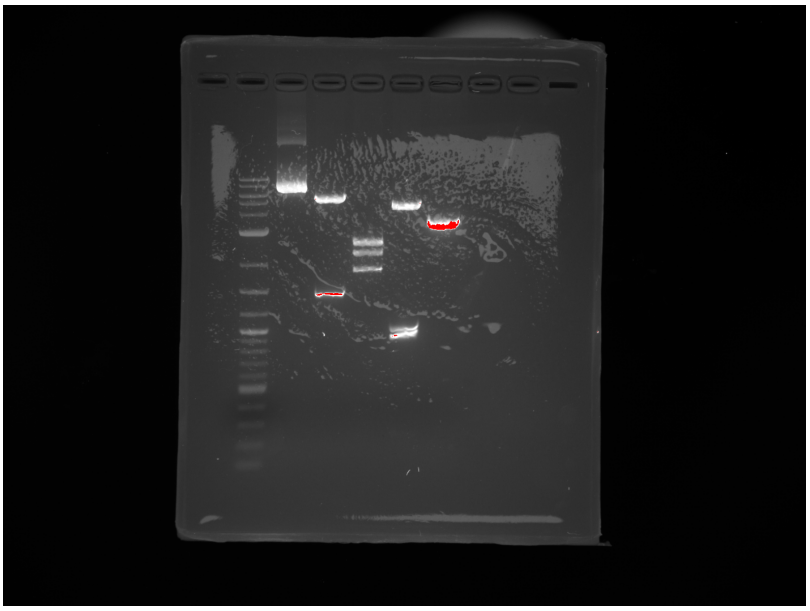
Gel 1: Singles

- Lane 1 – Empty
- Lane 2 – Ladder
- Lane 3 – Neg. Control
- Lane 4 – BamH1
- Lane 5 – Not1
- Lane 6 – Pac 1
- Lane 7 – Spe 1 + Cal1



Gel 2: Multiple

- Lane 1 – Empty
- Lane 2 – Ladder
- Lane 3 – Neg. Control
- Lane 4 – EcoR1 + BamH1
- Lane 5 – xBa1 + HindIII
- Lane 6 – EcoRI +Pst1
- Lane 7 – xHo1 + Spe1



Restriction Digest of Plasmids

- BamHI+EcoRI
- PacI
- BamHI
- NotI

15-16uL water
2uL SmartCut
1uq plasmid
1uL enzyme

Heat incubate 1-2 hours
Run gel

Big flask - bacterial contamination
Small - fungal
Threw out all media

Streaked humanized dcas9 on amp and 2x amp plates

June 24, 2016

What We Did Today:

Attended NEGEM Meeting #1